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Conformational Isomers of *Trans*-Urocanic Acid Observed by Rotational Spectroscopy

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ABSTRACT

Rotational spectra have been measured and assigned for four conformers of *trans*-urocanic acid. The acid was transferred into the gas phase through laser vaporisation of a solid sample, mixed with a neon buffer gas and then cooled through supersonic expansion. Molecules and complexes in the expanding gas jet were probed through chirped-pulse, Fourier transform microwave spectroscopy between 2.0 and 18.5 GHz. Rotational constants, A_0, B_0 and C_0; centrifugal distortion constants, Δ_J and Δ_{JK}; and nuclear quadrupole coupling constants of the nitrogen atoms, χ_{aa}(N) and χ_{bb}(N)−χ_{cc}(N), were determined for the various conformers. Data were obtained for ten isotopologues of the conformer that was observed to yield the spectrum of highest intensity. Substitution (r_s) coordinates were determined for all carbon atoms and two hydrogen atoms of this conformer. Other observed spectra were assigned to conformers on the basis of excellent agreement between calculated and experimentally-determined rotational constants, and empirical observations of the relative intensities of *a*- and *b*-type transitions. The results of DFT calculations imply high barriers to the interconversion of assigned conformers.

1. Introduction

Urocanic acid (UCA, (2*E*)-3-(1*H*-imidazol-4-yl)prop-2-enoic acid, shown in Figure 1) is an imidazole derivative produced in the *trans*-isomer by deamination of the naturally occurring amino acid histidine.[1-3] UCA builds up in the skin due to the absence of the urocanase enzyme,[4] and was previously believed to be a natural sunscreen compound, due to its absorption of UVB light in a process which stimulates *cis*-*trans* isomerisation.[3, 5, 6] Recent work has implicated the *cis*-isomer in the immune system suppression which is key to the development of skin cancers.[7, 8] The importance of UCA in biological processes has led to a large number of studies and reviews of this molecule and its photochemistry.[2-4] An attempt by Godfrey & Robertson to record a rotational spectrum of UCA was stymied by a facile decarboxylation reaction to produce 4-vinylimidazole which occurred in the heated source used.[9] The present work will show that laser vaporisation followed by cooling within a supersonically-expanding gas sample allows *trans*-UCA to be transferred into the gas phase for spectroscopic study. The geometries of four conformers of this molecule are determined through analyses of their broadband rotational spectra that are supported by Density Functional Theory (DFT) calculations. Substitution (r_s) coordinates of all carbon atoms are provided for the conformer determined to have the lowest energy.

A recent combined spectroscopic and computational study by Arjunan *et al*. provided insight into the structure of UCA in the solid state[10] but conformations of the molecule have not previously been isolated and structurally-characterised through gas phase spectroscopy. The *cis*- and *trans*-isomers of UCA are distinguished by different bonding configurations at the C(7) atom (using the atom labels illustrated in Figure 1). Simple inspection of the connectivity of *trans*-UCA suggests that conformational isomerism will also result from different steric configurations at the

C(4), C(8), and O(10) atoms. Further inferences can be drawn from previous works performed on molecules that have similar structural features. Similar molecules that have been the subject of previous microwave spectroscopic experiments include propenoic acid,[11] *trans*-2-butenoic acid,[12, 13] *trans*-cinnamic acid, and *trans*-*p*-coumaric acid.[14] Two low energy conformers, which differ in respect of the value of the dihedral angle, ∠(O=C–C=C) that determines whether the orientation of the carbonyl group is *cis* or *trans* to the alkene double bond, were structurally-characterised during the studies of each of the acids listed above. A low barrier to internal rotation of the CH_3 group (about the $H_3C–C_2H_2CO_2H$ bond) was identified from splittings observed in rotational transitions during the previous study of *trans*-2-butenoic acid.[13] Multiple conformers were observed in the spectra of each of *trans*-cinnamic acid and *trans*-*p*-coumaric acid.[14] The present work determines structural parameters for four conformers of *trans*-UCA that are distinguished from each other by their respective steric configurations at the C(4) and C(8) atoms. The results will be discussed with reference to the earlier studies of related molecules.

2. Experimental and theoretical methods

Initial results were obtained using a sample of *trans*-UCA (Sigma-Aldrich, 99%) containing naturally-occurring abundances of isotopes. Subsequent experiments to study deuterated isotopologues employed samples of *trans*-UCA enriched in deuterated forms through solution-phase proton exchange with D_2O (Sigma-Aldrich, 99.9 atom%) and DCl. Each solid sample was mixed with copper powder (Sigma-Aldrich, <75 μm, 99%) and a small amount of PVA glue, which serves as a binding agent, prior to being compressed into a 13 mm-diameter "target" rod which was subsequently used in laser vaporisation experiments. Each rod is prepared

to contain 5:3 *trans*-UCA:Cu by mass. The surface of the rod is ablated by the focussed pulse of a Nd:YAG laser (wavelength of 532 nm, pulse energy of ~15 mJ pulse^{-1}) such that vaporised material is entrained within a supersonic expansion of buffer gas introduced at a backing pressure of 7 bar from a pulsed valve. The data presented in Figure 2 were recorded while using neon as the buffer gas (BOC, CP grade) and these data were analysed to determine the spectroscopic parameters included in Tables 1, 3, 4 and 5. Experiments were also performed while using 7 bar of argon (BOC, CP grade) as buffer gas to provide insight into conformational relaxation within the probed sample (Section 3.3). A detailed description of the laser ablation source and chirped-pulse Fourier transform microwave (CP-FTMW) spectrometer used for the experiments has been published previously.[15-18]

Molecules within the expanding gas sample are polarised by a chirped microwave pulse of 1 μs in duration. The 2.0 – 8.0 and 7.0 – 18.5 GHz bands are probed during distinct experiments, each using a pulse that sweeps across the appropriate frequency band. The free induction decay (FID) of the molecular ensemble polarisation is recorded over 20 μs, beginning shortly after the microwave pulse. The time required for the gas pulse to traverse the spectrometer is significantly longer than the duration of a single FID so the measurement sequence described above is repeated eight times following each nozzle pulse. The experiments described herein were performed while employing a 2 Hz nozzle pulse repetition rate such that the spectrum displayed in Figure 2 was recorded over about 16 hours of real time. A 100 GS/s oscilloscope was used to digitise and record FIDs. A high resolution window function was used to perform the Fourier transformation to obtain peaks with estimated full-widths at half-maximum of ca. 100 kHz and an estimated peak frequency precision of ca. 10 kHz. An arbitrary waveform generator, phase-locked dielectric resonant

oscillator and oscilloscope are locked to a Rb clock, which provides a 10 MHz reference that allows coherent averaging of the data in the time domain.

Density Functional Theory calculations were performed using the Gaussian09[19] software package in order to optimise the geometries and compare the relative energies of conformers of urocanic acid. The B3LYP functional was used,[20-23] with Grimme's empirical dispersion correction with function damping (D3BJ),[24, 25] in conjunction with Dunning's augmented triple zeta aug-cc-pVTZ basis set.[26-29] The same functionals and basis sets were also used to scan the potential energy surfaces along internal rotation coordinates for *trans*-UCA and *trans*-2-butenoic acid. These were performed as unrelaxed scans (where the molecular skeleton was held rigid) where the coordinate scanned was the dihedral angle, ∠(O=C–C=C), which determines whether the orientation of the carbonyl group is *cis* or *trans* to the alkene double bond. Relaxed scans were performed (with coordinates except the dihedral angle in question being allowed to optimise) when scanning over the coordinate describing rotation of the $-CH_3$ or imidazolyl terminal groups, respectively, relative to the rest of the molecule. Atomic coordinates calculated by DFT for each conformer are provided in Tables S1-S4 with the results of DFT calculations of spectroscopic parameters available in Table S5.

3. Results and discussion

3.1 Spectral assignment and analysis

The spectrum recorded under the experimental conditions described in Section 2 is displayed in Figure 2. Separate experiments were performed to measure the spectrum across the

2.0 – 8.0 GHz and 7.0 – 18.5 GHz ranges where the data were averaged over 5.04 and 6.81 million FIDs respectively. Further to transitions which will be shown to assign to urocanic acid, products of the fragmentation of the parent molecule are amongst the species observed. Many small molecules, for which microwave spectra have previously been reported, are confirmed as present through their characteristic transition frequencies. These include C_4H_5N (imidazole),[30] HC_3N,[31-33] HC_5N,[34] HC_7N,[35] CH_3CN,[36] CH_3CCCN,[37, 38] and vinyl cyanide (CH_2=CHCN).[39-41] A tendency for the described experimental conditions to generate products of the fragmentation of precursors has been noted during recent studies of imidazole and pyrazole derivatives.[18, 42, 43] Godfrey *et al.* attempted to transfer urocanic acid into the gas phase through heating but noted that decarboxylation tended to cause fragmentation of the precursor to yield 4-vinylimidazole. It is therefore unsurprising that the two lowest energy conformers of 4-vinylimidazole[9] are present in the spectra acquired during the present work. Trace quantities of water, present as a contaminant of the gas sample, explain the presence of transitions that assign to $(H_2O)_2$.[44] Transitions of these fragmentation products and contaminants have been removed from the spectrum displayed in Figure 2. Isotopically-enriched samples were used to allow recording of the spectra of deuterated isotopologues. The spectrum recorded while using a deuterated sample of UCA in the 7.0 – 18.5 GHz region was averaged over 2.13 million FIDs and several new features were observed. Some of these readily assign to deuterated fragmentation products, specifically DC_3N,[45] CH_2DCN,[46, 47] and CHD_2CN.[47, 48] It will be shown that other transitions assign to the spectra of deuterated isotopologues of conformers of *trans*-UCA. Spectra were carefully surveyed for evidence of transitions of *cis*-UCA, which differs from *trans*-UCA in respect of the steric configuration at the C(7) atom, but no evidence of this isomer was identified.

Measured transition frequencies were assigned with quantum numbers and initial, approximate fits of spectroscopic parameters were performed using PGOPHER.[49-51] The results were finalised using Pickett's SPFIT.[52] The Hamiltonian used for the fits was:

$$H = H_{\mathrm{R}} - \tfrac{1}{6}\mathbf{Q}(\mathrm{N1}):\nabla\mathbf{E}(\mathrm{N1}) - \tfrac{1}{6}\mathbf{Q}(\mathrm{N3}):\nabla\mathbf{E}(\mathrm{N3}), \qquad (1)$$

where H_{R} is the energy operator for a semi-rigid asymmetric rotor in Watson's *A*-reduction, and the remaining terms represent interactions between the nuclear electric quadrupole moment and the molecular electric field gradient at each nitrogen nucleus (numbered as in Figure 1). H_{R} is comprised of terms dependent on the A_0, B_0 and C_0 rotational constants, and Δ_J, Δ_{JK} , centrifugal distortion constants. The nuclear quadrupole moment dyadic is represented by **Q** with the dyadic of the electric field gradient being denoted by $\nabla\mathbf{E}$. Components of the nuclear quadrupole coupling tensor of each quadrupolar nitrogen nucleus are denoted by χ_{aa} and $(\chi_{bb} - \chi_{cc})$. The matrix elements were constructed in the coupled asymmetric rotor basis, $\boldsymbol{J} + \boldsymbol{I}_{\mathrm{N1}} = \boldsymbol{F}_1$, $\boldsymbol{F}_1 + \boldsymbol{I}_{\mathrm{N3}} = \boldsymbol{F}$, and diagonalised in blocks of the quantum number, *F*. Hyperfine splittings introduced by the hydrogen or deuterium nuclei were not resolved during these experiments.

Conformers of *trans*-UCA that are differentiated by their steric configuration at the C(6), C(8) and/or O(10) atoms all have geometries that are near-prolate asymmetric rotors and each has a molecular dipole moment that is nearly aligned with the *a*-inertial axis. It was therefore anticipated that each spectrum of a conformer of *trans*-UCA would be characteristic of a near-prolate asymmetric rotor and have prominent *a*-type transitions. The results of the DFT calculations provided approximate values of A_0, B_0 and C_0 rotational constants of eight conformers which will be further discussed in Section 3.3. The spectra of four different species, which each satisfied all of the expectations described above, were identified in the experimental data and it

will be shown that these assign to the four conformers of *trans*-UCA which are calculated to have the lowest energy. The four spectra identified are hereafter labelled using Roman numerals with increasing number correlating with decreasing spectral intensity although III and IV are actually of broadly similar intensity. II and III include some *b*-type transitions of weaker intensity than the *a*-type transitions observed of the same species. The fitted values of spectroscopic parameters are given in Table 1.

Hyperfine splittings were observed and assigned for I-III, leading to the determination of values of χ_{aa} and $(\chi_{bb} - \chi_{cc})$. The values of hyperfine parameters are especially sensitive to transitions involving lower J which were detected only with weak intensity for IV, precluding the determination of nuclear quadrupole coupling constants for this conformer. A value of χ_{aa}(N3) could not be determined for II, presumably because the introduced hyperfine splittings are too small to be resolved by the present experiments. The values of these parameters were therefore fixed to the values of the calculated results. After the appropriate transformation from the inertial axis framework into frames referenced against principal nuclear axes of the nuclear quadrupole coupling tensor, evaluated nuclear quadrupole coupling constants of the nitrogen atoms are very similar to those previously determined for the isolated imidazole molecule. Values of χ_{xx}, χ_{yy} and χ_{zz} (where x, y and z are the principal axes of the nuclear quadrupole coupling tensor) obtained through the described transformation are provided within the Supplementary Data (Table S6) alongside results for other molecules that contain an imidazole ring.

Further evidence that the observed spectra should be assigned to conformers of *trans*-UCA is presented in Section 3.2 where the molecular geometries of the various conformers are examined in detail. Noting that proton exchange is known to occur between the pyrrolic and pyridinic nitrogens of imidazole,[53, 54] spectra were searched for evidence of isomers where the propenoic

acid group is attached to C(5) of the imidazole ring. No evidence for such isomers was observed. This suggests that proton exchange is not effective under the present experimental or sample preparation conditions, consistent with a previous observation of the spectrum of 4-iodoimidazole.[43]

3.2 Characterisation of conformer geometries

Rotational constants, A_0, B_0 and C_0, were determined for all spectra identified which allows for the calculation of inertial defects and planar moments for each of the assigned conformers. The significance of the inertial defect, Δ_0, is that small, positive values are typically associated with planar molecules. For example, the values of Δ_0 for imidazole[30] and pyrazole[55] are 0.02865(26) and 0.0321(3) uÅ^2 respectively. The inertial defect is calculated as follows:

$$\Delta_0 = I_c^0 - I_b^0 - I_a^0 \qquad (2)$$

where I_a^0, I_b^0 and I_c^0 are the moments of inertia determined from the A_0, B_0 and C_0 rotational constants included in Table 1. Values of Δ_0 (shown in Table 1) are between –0.48276(87) and –0.7778(31) uÅ^2 for each of the molecular carriers of the spectra which are assigned here to conformers of *trans*-UCA. A negative value of Δ_0 = –0.05 uÅ^2 was cited as evidence of a planar geometry for propenoic acid.[11] The inertial defect of *trans*-2-butenoic acid,[13] which is non-planar by virtue of its CH_3 group, was determined to be –3.18 uÅ^2 which is significantly more negative than the inertial defects of all species identified herein. Vibrational motions are known to make a significant contribution to inertial defects. In general, in-plane vibrations contribute positively to Δ_0 while out-of-plane vibrations make negative contributions. Low frequency torsional motions

can be expected to be a feature of the internal dynamics of *trans*-UCA. Further insight is provided by the planar moments which are calculated as:

$$P_\alpha = \frac{1}{2}\left(-I_\alpha + I_\beta + I_\gamma\right) \tag{3}$$

where α, β and γ are permuted cyclically over the inertial axes and it can be noted that $P_c = -\frac{1}{2}\Delta_0$. There is little variation in the values of each parameter, P_a, P_b and P_c, when comparing the results fitted to different spectra but the value of P_a is consistently greater than that of P_b or P_c for all species identified. Small, positive values of P_c imply molecules that are effectively planar in the ground vibrational state with low frequency, out-of-plane vibrations significantly contributing to both planar moments and inertial defects. Consistent with the narrow range of values of each of P_a, P_b and P_c, determined for I–IV, altering the steric configurations at the C(4) or C(8) atoms of *trans*-UCA will re-orient groups without significantly changing the overall near-prolate shape or planar geometry of the molecule. The asymmetry parameter,[56] κ is determined to be –0.97 for all conformers identified. The observations are therefore consistent with the proposal that the four conformers A-D illustrated in Figure 3, which are the four conformers of lowest energy, assign to the spectra labelled I-IV in Table 1.

The results of DFT calculations inform further analysis and more specific assignments of conformers to the observed spectra. The equilibrium geometries of conformers of urocanic acid were optimised to determine rotational constants, dipole moments and relative energies as shown in Table 2. The inertial defect, Δ_e, is calculated to be zero for each conformer as expected for planar equilibrium geometries. The optimisation allowed for relaxation of each dihedral angle describing rotation about a single bond within *trans*-UCA. The four conformers illustrated in

Figure 3 are those calculated to have the lowest energy; other conformers lie at least ca. 30 kJ mol^{-1} above the global minimum. The spectroscopic parameters determined from the experimental data (Table 1) will be those appropriate to the ground vibrational states of the various conformers. The spectra of vibrationally-excited conformers were not apparent within the experimental data. However, the rotational constants determined from the experimental data are very similar to those calculated for the equilibrium geometries of conformers A-D (Table 2). The high level of agreement between the calculated rotational constants for conformer A and those determined experimentally for I imply that this spectrum should be assigned to conformer A. The same logic suggests that II, III and IV should be assigned to conformers B, C and D, respectively. It is encouraging that this assignment yields very good agreement between all calculated and experimentally-determined rotational constants. The remainder of this work will therefore proceed on the basis of the assignment described above and present further evidence in support of this proposal.

The evidence is strongest in respect of conformer A where the spectra of many isotopologues that contain ^{13}C were measured. These were observed between 7.0 and 18.5 GHz while using samples containing isotopes in natural abundance. Fitted spectroscopic parameters of six isotopologues of conformer A, that each contain ^{13}C, are displayed in Table 3. Substitution of deuterium for hydrogen was achieved at the pyrrolic nitrogen atom of the imidazole ring and within the acid group of *trans*-UCA. The results of fitting the rotational and centrifugal distortion constants of deuterated isotopologues are given in Table 4. Spectra of conformer A were assigned for all three permutations of deuterated isotopologue that can result from substitution at the above sites. Kraitchman's equations, which allow the calculation of coordinates in the r_s geometries of the molecule, were applied to determine the positions of carbon and hydrogen atoms using Kisiel's

program, KRA, available from the PROSPE repository.[57, 58] The determined coordinates are presented in Table 5 alongside DFT calculated results for conformer A. There is good agreement in every case. The discrepancies between values are somewhat larger when the atoms lie close to the *a*- or *b*-axes, as expected, because the method is known to be inaccurate with respect to small coordinates. Significant zero-point vibrational effects also introduce significant inaccuracies into hydrogen atom coordinates calculated using Kraitchman's equations.

The positions of individual hydrogen atoms could not be determined for conformer B because data are not available for an isotopologue where only one hydrogen atom is substituted for deuterium. However, the values of A_0, B_0, and C_0 determined for a doubly-deuterated isotopologue of this conformer agree with calculated values of the same parameters, which are 4831.58, 560.26, and 502.05 MHz respectively. Data were obtained for only one isotopologue of each of conformers C and D so atomic substitution coordinates are not available for these conformers either. In these cases, the assignment of conformers to spectra is principally on basis of the close agreement between rotational constants *ab initio* calculated for these conformers and parameters determined experimentally. However, further evidence that the assignments are correct is apparent in the types of transitions observed. Spectra assigned to conformers B and C include *b*-type transitions while those assigned to conformers A and D do not. This is consistent with values of the *b*-component of dipole moments, $|\mu_b|$, which are calculated to be significantly larger for conformers B and C than for conformers A and D. Some transitions in the observed spectra were not assigned to conformers A–D but could be fitted to determine the spectroscopic parameters of a hitherto unidentified species. The planar moments of this species are too large for the molecular carrier to be another conformer of *trans*-UCA. Instead, it seems likely that the molecular

carrier is a complex of *trans*-UCA and H_2O. Ongoing experiments are exploring this possibility while using isotopically-enriched samples.

3.3. Discussion

Many studies have explored the interplay between intramolecular forces and molecular geometry within carboxylic acids. An important theme of recent work has been the effect of fluorination on conformation.[59-62] The range of conformers identified for *trans*-UCA can be rationalised through comparison with the results of previous studies of conformation in similar molecules containing the propenoic acid group.[11, 13, 14]

The low energy conformers of *trans*-UCA, illustrated in Figure 3 and respectively assigned to spectra as described above, each have a geometry in which the orientation of the O–H bond is *cis* to the carbonyl oxygen atom. The observations are thus consistent with studies of propanoic acid and four propenoic acid derivatives (propenoic, *trans*-2-butenoic, *trans*-cinnamic, and *trans*-*p*-coumaric acids), where conformers observed by rotational spectroscopy were exclusively *cis*-OH isomers.[11, 13, 14, 63] The DFT calculations of the present work identified four conformers (E-H) of higher energy than conformers A-D through geometry optimisation. While conformers A-D are *cis*-OH, conformers E-H are *trans*-OH and do not readily assign to any spectra observed during the present work. The higher energies of *trans*-OH isomers presumably result from steric repulsion of the hydrogen atom on the α-carbon. The two observed conformers of each of the four derivatives of propenoic acid listed above differ in respect of the dihedral angle, ∠(O=C–C=C), which determines whether the orientation of the carbonyl group is *cis* or *trans* to the alkene double bond (hereafter labelled as *s-cis* or *s-trans* to follow the notation used in the earlier study of *trans*-2-butenoic acid). The *s-cis* and *s-trans* conformers of propenoic acid were observed in similar

abundance within the sample probed during the previous study of the molecule. Calculations performed during the earlier study of *trans*-2-butenoic acid found the energy of the *s-cis* conformer to be 0.95 kcal mol^{-1} (4.0 kJ mol^{-1}) lower than that of the *s-trans* conformer. Similar calculations on *trans*-cinnamic acid and *trans-p*-coumaric acid found *s-cis* conformers to be lower in energy than *s-trans* conformers by 191-215 cm^{-1} (2.3 to 2.6 kJ mol^{-1}). The DFT calculations of the present work confirm that the energy ordering of *s-cis* (conformers A and C) and *s-trans* (conformers B and D) isomers of *trans*-UCA is consistent with the *s-cis*/*s-trans* energy ordering determined for the related molecules listed above.

An experiment performed during the present work confirmed that spectra are observed for conformers A and B of *trans*-UCA (which are respectively *s-cis* and *s-trans*), with the S/N ratio of the most intense transitions of each conformer being greater than 50, even when argon is used as the buffer gas. Some of the most intense peaks in the spectra of conformers C and D were also observed under these conditions. Relaxation to yield low energy conformers generally proceeds more efficiently when the buffer gas employed is argon rather than neon.[64] Evidently, conformers B, C and D of *trans*-UCA retain a significant population even where argon buffer gas is employed, implying a significant barrier to relaxation to lower energy conformers. During the present work, calculations of the height of the barrier that impedes relaxation of the ∠(O=C–C=C) angle (where the conversion is from the *s-trans* conformer to the *s-cis* conformer) yielded 27 and 30 kJ mol^{-1} for *trans*-2-butenoic acid and *trans*-UCA respectively. On the basis of rotational transition intensities, the previous study of *trans*-2-butenoic acid concluded that the *s-cis* conformer of this molecule was present in higher proportion than the *s-trans* conformer within the sample probed. The same logic would imply that conformer A (an *s-cis* conformer) of *trans*-UCA is more abundant than conformer B within the probed sample but the data do not allow for more quantitative

conclusions in this respect. The barrier to interconversion between the *s-cis* and *s-trans* isomers of propenoic or *trans*-2-butenoic acid is the energy required to disrupt conjugation across the alkene and carbonyl double bonds. In the case of propanoic acid, where no such conjugation exists, the barrier to relaxation of the higher energy, *s-trans* conformer to yield the *s-cis* conformer was predicted by an unrelaxed DFT scan to be ca. 1.4 kJ mol^{-1}, which is similar to MP2 results provided by Jaman *et al*.[65] The *s-cis* form of each of propanoic and trifluorobutyric acids were observed during microwave spectroscopic experiments to study these saturated-chain carboxylic acids.[62, 63, 65]

The barrier to internal rotation of the CH_3 group in *trans*-2-butenoic acid arises from minor steric effects that are a result of methyl protons eclipsing the hydrogen atom on a neighbouring carbon atom during internal rotation. The associated barrier was determined computationally to be 7.78 kJ mol^{-1} for both *s-cis* and *s-trans* conformers, consistent with experimental values of 1.83(10) kcal mol^{-1} (7.66(42) kJ mol^{-1}) and 1.754(88) kcal mol^{-1} (7.34(37) kJ mol^{-1}) respectively. Rotation of the terminal methyl group in *trans*-2-butenoic acid[12] was observed to yield splittings of rotational transitions. Conformers A and B of *trans*-UCA are collectively distinguished from conformers C and D by the value of the dihedral angle, ∠(C(7)=C(6)-C(4)-N(3)), which is 0° for conformers A and B and 180° for conformers C and D. The barrier that hinders the conversion of conformer A to conformer C is calculated to be 36.95 kJ mol^{-1} while that which hinders the conversion of conformer B to D is calculated to be 35.55 kJ mol^{-1} respectively. Evidently, extended conjugation between the aromatic ring and the π-system in the propenoic acid group of *trans*-UCA means that the energy barrier encountered when varying the ∠(C(7)=C(6)-C(4)-N(3)) dihedral angle (ie, when rotating the imidazole ring relative to the rest of the molecule) is significantly greater than is the 3-fold barrier to internal rotation in *trans*-2-butenoic acid. The isolation and

observation of four different conformers of *trans*-UCA also implies that relaxation to the lowest energy conformers does not proceed efficiently, particularly where neon is the buffer gas employed, under the experimental conditions of the present work. A previous study of *trans-p*-coumaric acid identified conformers distinguished by the relative orientations of propenoic acid and hydroxyl groups on this molecule.[14] The difference in energy between *syn*- and *anti*-configurations of these groups was found to range from 14 to 38 cm^{-1} (0.17 – 0.46 kJ mol^{-1}). This is significantly lower than the energy difference between conformers A and C, or of that between conformers B and D, as calculated for *trans*-UCA during the present work. The range of four conformers observed during the previous study[14] implies that relaxation to the lowest energy conformer of *trans-p*-coumaric acid also proceeds inefficiently where neon is employed as buffer gas.

4. Conclusions

The pure rotational spectrum of urocanic acid has been recorded between 2.0 and 18.5 GHz. Spectroscopic parameters, including rotational and centrifugal distortion constants, have been determined for the four conformers that are calculated by DFT to have the lowest energy. Substitution coordinates (r_s) have been determined for all carbon atoms and two hydrogen atoms within the lowest energy conformer. The observed conformers all adopt geometries in which the orientation of the O–H bond is *cis* to the carbonyl oxygen atom. With respect to the orientation of the acid group relative to the rest of the molecule, the observed isomerism is analogous to that observed previously for other propenoic acid derivatives such that conformers are observed in both *s-cis* and *s-trans* arrangements. The values of nuclear quadrupole coupling constants, χ_{xx}, χ_{yy} and χ_{zz} are similar to those previously identified in other molecules that contain an imidazole ring. This work has focussed on *trans*-UCA which is significantly more readily obtainable than *cis*-UCA.

However, the observations of the present work imply that distinct conformational isomers of *cis*-UCA may be isolated and studied within a supersonically-expanding gas sample.

Conflicts of Interest

There are no conflicts of interest to declare.

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FIGURES:

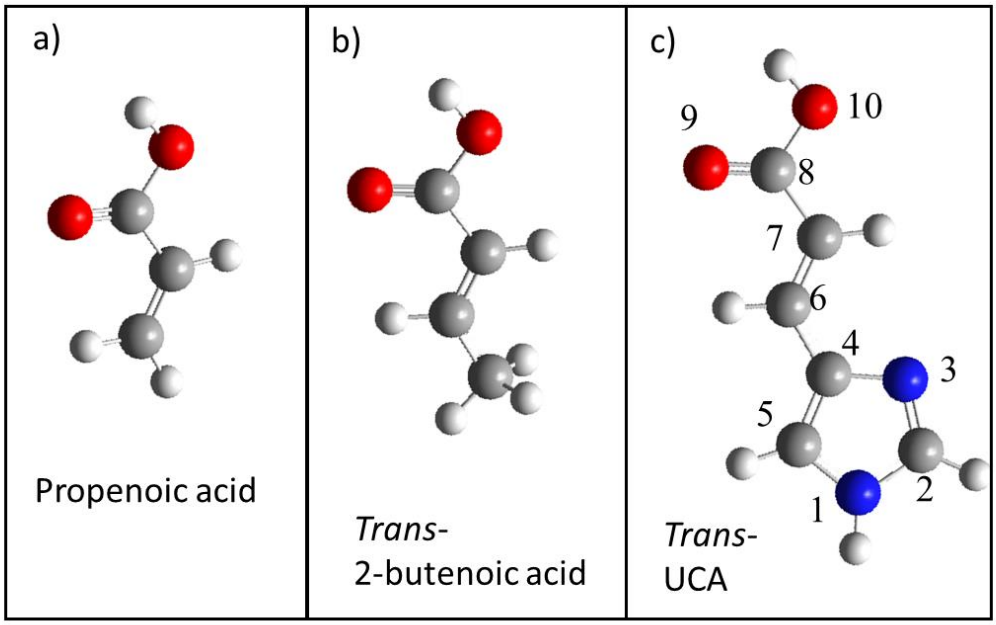

Figure 1. Geometries of (a) propenoic acid, (b) *trans*-2-butenoic acid and (c) *trans*-UCA. Heavy atoms of *trans*-UCA are numbered to allow the explicit definition of geometrical coordinates within the text.

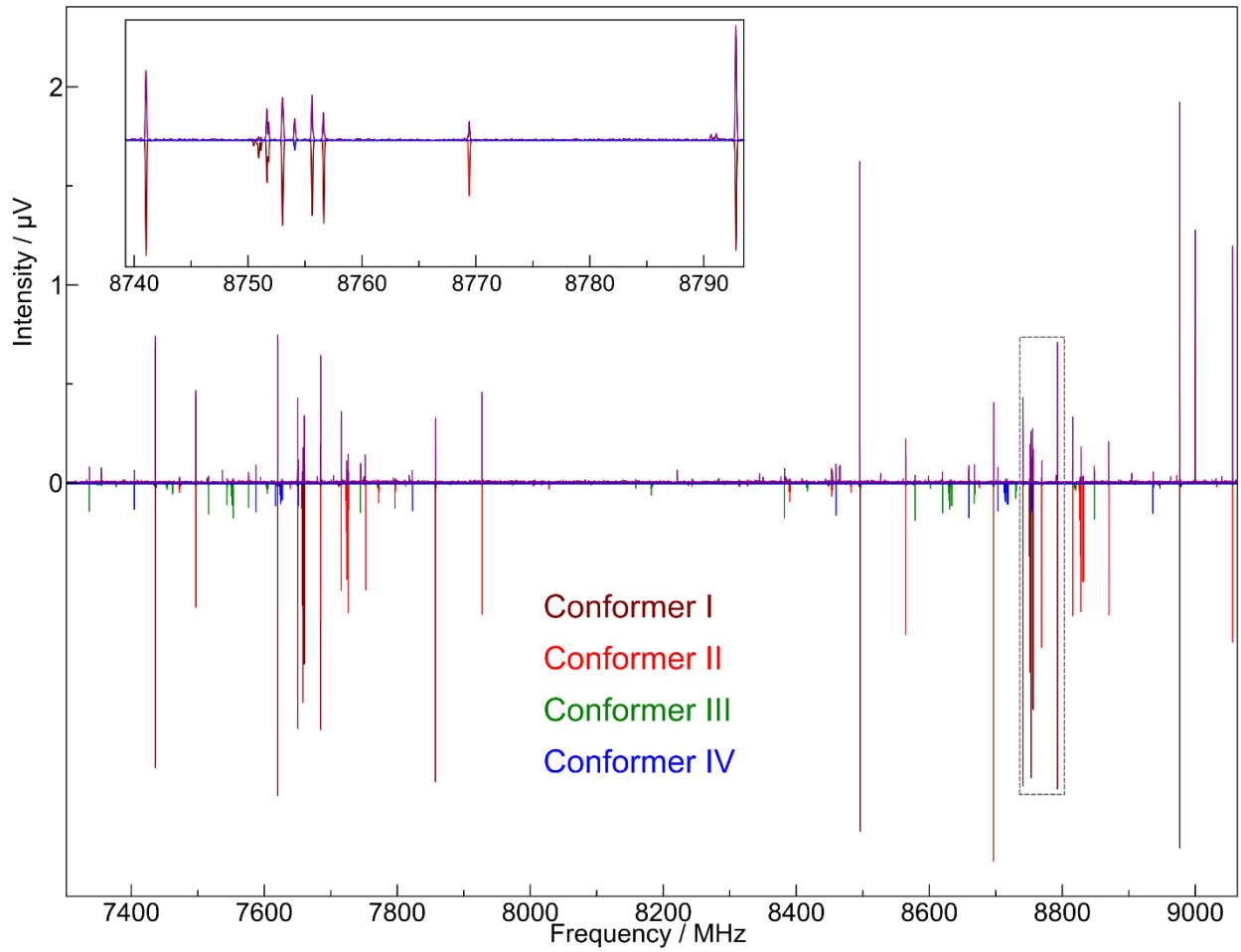

Figure 2. Microwave spectrum of urocanic acid displaying $J' \rightarrow J'' = 7 \rightarrow 6$ and $8 \rightarrow 7$ transitions respectively. The experimental data are on the top, with the simulations produced by PGOPHER from the constants in Table I on the bottom. **Inset:** Detail of the $J' \rightarrow J'' = 8 \rightarrow 7$ transition of spectrum I, corresponding to the region in the dashed grey box in the main figure.

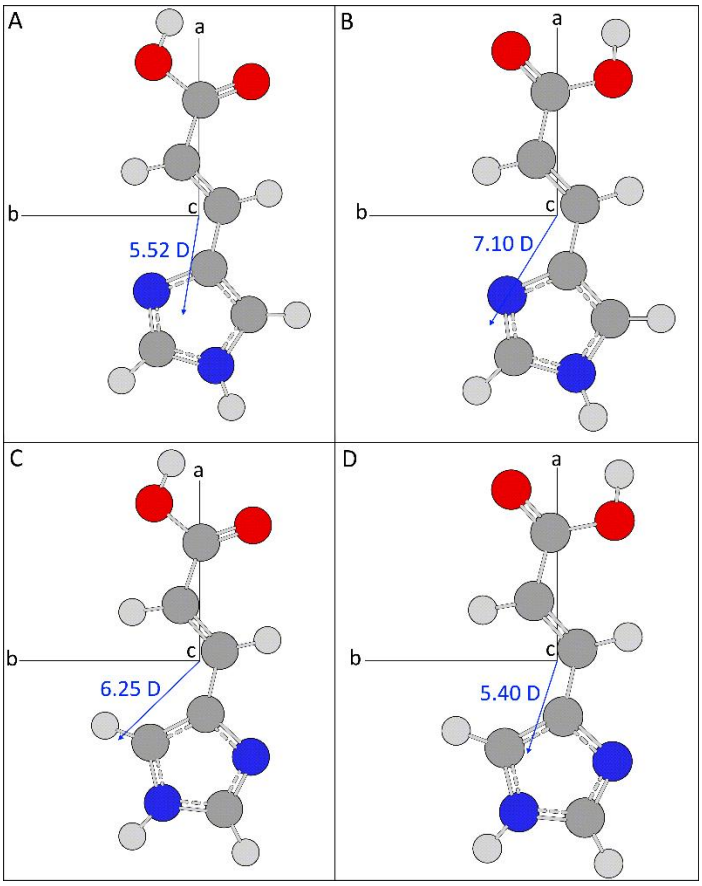

Figure 3. The geometries of the four lowest-energy conformers of urocanic acid as predicted using B3LYP-D3BJ/aug-cc-pVTZ calculations in Gaussian 09. The inertial axes are indicated for each conformer, with the *c*-axis coming out of the page; the centre of mass is at the origin of each axis system. Arrows indicate the orientation and relative magnitude of the dipole moment for each species.

Table 1. Experimental values of ground-state spectroscopic constants of conformers of *trans*-UCA (all values from SPFIT; numbers in brackets indicate standard deviations in units of last digit).

Spectroscopic Constant[a]	I	II	III	IV
A_0 / MHz	4920.844(15)	4899.57069(52)	4932.59263(97)	4907.69(10)
B_0 / MHz	576.93690(17)	582.26973(17)	568.35033(18)	574.28994(27)
C_0 / MHz	516.64802(18)	520.69877(14)	510.02466(13)	514.53661(29)
Δ_J / kHz	0.00819(26)	0.00882(39)	0.00822(32)	0.00782(43)
Δ_{JK} / kHz	0.0183(12)	0.0298(24)	0.0164(80)	0.0508(45)
χ_{aa} (N1) / MHz	1.321(14)	1.316(16)	1.303(36)	[1.273][b]
$[\chi_{bb}(N1)-\chi_{cc}(N1)]$ / MHz	4.03(26)	3.707(18)	3.499(34)	[3.570][b]
χ_{ab} (N1) / MHz	[0.032][b]	[0.031][b]	[–0.096][b]	[–0.090][b]
χ_{aa} (N3) / MHz	–0.134(33)	[–0.014][b]	1.341(35)	[1.448][b]
$[\chi_{bb}(N3)-\chi_{cc}(N3)]$ / MHz	–4.72(36)	–4.275(18)	-6.000(33)	[–6.499][b]
χ_{ab} (N3) / MHz	[–2.934][b]	[–2.906][b]	[1.422][b]	[1.517][b]
P_A	875.7074(5)	867.6684(4)	888.7980(4)	879.595(2)
P_B	102.458(5)	102.8873(4)	102.0701(4)	102.584(2)
P_C	0.2414(5)	0.2579(4)	0.3845(4)	0.391(2)
Δ_0[c]	–0.483	–0.516	–0.769	–0.781
κ[d]	–0.973	–0.972	–0.974	–0.973
N[e]	190	303	139	83
$\sigma_{r.m.s.}$ / kHz[f]	10.0	16.6	20.5	12.2

[a] Fit performed using Watson's A reduced Hamiltonian
[b] Value fixed at result calculated for assigned geometry.
[c] $\Delta_0 = I_c - I_b - I_a$
[d] $\kappa = (2B_0 - A_0 - C_0)/(A_0 - C_0)$
[e] Number of transitions included in fit.
[f] Root mean square deviation of fit.

Table 2. Calculated rotational constants and dipole moment components for ground-state geometries of different conformers of *trans*-UCA (calculated at the B3LYP-D3BJ/aug-cc-pVTZ level in Gaussian 09)

Conformer	*A* / MHz	*B* / MHz	*C* / MHz	$\|\mu_a\|$ / D	$\|\mu_b\|$ / D	E / kJ mol^{-1} [a]
A	4953.4348	576.6934	516.5546	5.45	0.91	0.0
B	4931.6187	582.0086	520.5728	6.05	3.72	2.8
C	4966.4367	568.8648	510.4024	4.37	4.47	6.8
D	4942.4948	574.7739	514.8955	5.14	1.66	7.4

[a] Relative to global minimum.

Table 3. Experimental values of ground-state spectroscopic constants of ^{13}C isotopologues of conformer I of *trans*-UCA (all values from SPFIT; numbers in brackets indicate standard deviations in units of last digit).

Spectroscopic Constant[a,b]	2-^{13}C	4-^{13}C	5-^{13}C	6-^{13}C	7-^{13}C	8-^{13}C
A_0 / MHz	4881.41(79)	4918.3(10)	4849.4(17)	4906.38(76)	4912.9(12)	4913.9(21)
B_0 / MHz	571.0602(34)	576.0353(10)	573.6941(15)	576.9256(10)	576.01555(90)	572.5131(12)
C_0 / MHz	511.5857(14)	515.89936(89)	513.3941(12)	516.4885(10)	515.82977(85)	513.1027(12)
N[c]	6	9	10	9	9	10
$\sigma_{r.m.s.}$ / kHz[d]	19.4	20.4	25.1	20.7	18.4	18.8

[a] Watson's A reduction

[b] These fits were performed while other parameters were fixed at their values determined for the most naturally-abundant isotopologue of conformer A (Table 1, Spectrum I) as follows; Δ_J=0.00819 kHz; Δ_{JK} = 0.0183 kHz; χ_{aa} (N1) = 1.321 MHz; [χ_{bb}(N1)–χ_{cc}(N1)] = 4.04 MHz; χ_{ab} (N1) = 0.032 MHz; χ_{aa} (N3) = –0.134 MHz; [χ_{bb}(N3)–χ_{cc}(N3)] = –4.73 MHz; χ_{ab} (N1) = –2.934 MHz.

[c] Number of transitions included in fit.

[d] Root mean square deviation of fit.

Table 4. Experimental values of ground-state spectroscopic constants of observed deuterated isotopologues of *trans*-UCA (all values from SPFIT; numbers in brackets indicate standard deviations in units of last digit).

Spectroscopic		I		II
Constant[a]	N-D	O-D	N-D + O-D	N-D + O-D
A_0 / MHz	4898.141(91)	4900.600(77)	4878.977(65)	4804.910(78)
B_0 / MHz	564.94733(24)	564.77642(20)	553.10306(16)	560.49304(21)
C_0 / MHz	506.77630(26)	506.66571(21)	497.03123(16)	502.21006(24)
Δ_J / kHz	0.00797(40)	0.00768(34)	0.00710(24)	0.00777(33)
Δ_{JK} / kHz	0.0216(80)	[0.0183][b]	0.0107(47)	0.0172(47)
χ_{aa} (N1) / MHz	[1.321][b]	[1.321][b]	[1.321][b]	[1.315][c]
χ_{bb-cc} (N1) / MHz	[4.04][b]	[4.04][b]	[4.04][b]	[3.706][c]
χ_{ab} (N1) / MHz	[0.032][b]	[0.032][b]	[0.032][b]	[0.031][c]
χ_{aa} (N3) / MHz	[–0.134][b]	[–0.134][b]	[–0.134][b]	[–0.014][c]
χ_{bb-cc} (N3) / MHz	[–4.73][b]	[–4.73][b]	[–4.73][b]	[–4.278][c]
χ_{ab} (N3) / MHz	[–2.934][b]	[–2.934][b]	[–2.934][b]	[–2.906][c]
N[d]	67	75	86	68
$\sigma_{r.m.s.}$ / kHz[e]	10.1	9.0	7.7	8.1

[a] Watson's A reduction
[b] Parameters are fixed at the values determined for the most naturally-abundant isotopologue of conformer A (Table 1, spectrum I).
[c] Parameters are fixed at the values determined for the most naturally-abundant isotopologue of conformer B (Table 1, spectrum II).
[d] Number of transitions included in fit
[e] Root mean square deviation of fit

Table 5. Substitution coordinates of atoms in the ground state conformation (conformer A) of *trans*-UCA (numbers in brackets indicate uncertainties including Costain errors[56, 66] of δa = 0.0015/|*a*| in units of the last digit)

Atom	*a* / Å		*b* / Å	
	Expt.[a]	Comp.[b]	Expt.[a]	Comp.[b]
C(2)	–3.0065(10)	–2.9905	0.9179(94)	0.9424
C(4)	–1.1732(14)	–1.1823	–0.231(46)	–0.219
C(5)	–2.22721(85)	–2.22106	–1.236(15)	–1.124
C(6)	0.131(13)	0.225	–0.551(15)	–0.519
C(7)	1.1858(14)	1.2062	0.409(31)	0.391
C(8)	2.60634(68)	2.60848	–0.383(58)	–0.036
H(1)[c]	–4.31225(35)	–4.30769	–0.6985(26)	–0.7263
H(10)[c]	4.34368(35)	4.35218	0.6596(26)	0.6674

[a] All calculations were performed while using the most naturally abundant isotopologue of conformer A (data provided in Table 1, spectrum I) as the "parent" isotopologue. The analysis employed Kraitchman's equations for a planar molecule.[57]
[b] From Gaussian 09 at the B3LYP-D3BJ/aug-cc-pVTZ level.
[c] Hydrogen atoms are labelled with the number of the heavy atom to which they are attached (see Figure 1).

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